

# Selection of a Water-Extractable Phosphorus Test for Manures and Biosolids as an Indicator of Runoff Loss Potential

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The correlation of runoff phosphorus (P) with water-extractable phosphorus (WEP) in land-applied manures and biosolids has spurred wide use of WEP as a water quality indicator. Land managers, planners, and researchers need a common WEP protocol to consistently use WEP in nutrient management. Our objectives were to (i) identify a common WEP protocol with sufficient accuracy and precision to be adopted by commercial testing laboratories and (ii) confirm that the common protocol is a reliable index of runoff P. Ten laboratories across North America evaluated alternative protocols with an array of manure and biosolids samples. A single laboratory analyzed all samples and conducted a separate runoff study with the manures and biosolids. Extraction ratio (solution:solids) was the most important factor affecting WEP, with WEP increasing from 10:1 to 100:1 and increasing from 100:1 to 200:1. When WEP was measured by a single laboratory, correlations with runoff P from packed soil boxes amended with manure and biosolids ranged from 0.79 to 0.92 across all protocol combinations (extraction ratio, filtration method, and P determination method). Correlations with P in runoff were slightly lower but significant when WEP was measured by the 10 labs ( $r = 0.56\text{--}0.86$ ). Based on laboratory repeatability and water quality evaluation criteria, we recommend the following common protocol: 100:1 extraction ratio; 1-h shaking and centrifuge 10 min at  $1500 \times g$  (filter with Whatman #1 paper if necessary); and determining P by inductively coupled plasma-atomic emission spectrometry or colorimetric methods.

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**A**CCCELERATED FRESHWATER EUTROPHICATION, the biological aging of surface waters related to anthropogenic enrichment of nutrients, is primarily controlled by inputs of phosphorus (P). Phosphorus-laden runoff from manure-amended soils is an important source of P in many eutrophic water bodies in North America (Carpenter et al., 1998). Application of livestock manures to soils can exacerbate P losses in runoff indirectly by augmenting soil P saturation and P desorption potential (Sibbesen and Sharpley, 1997) or directly through the transfer of recently applied manure P to runoff water (Preedy et al., 2001). As a result, there has been widespread implementation of P-based guidelines in the USA and Canada aimed at improving manure application practices to soils (USEPA, 2004; Hilborne and Stone, 2005), with a key strategy being the use of P site

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**Abbreviations:** ICP, inductively coupled plasma;  $P_{\text{color}}$ , phosphorus determined by colorimetry;  $P_{\text{ICP}}$ , phosphorus determined by inductively coupled plasma-atomic emission spectrometry; RSD, relative standard deviation; TP, total phosphorus; WEP, water-extractable phosphorus.

assessment indices to minimize application of manure to fields prone to P runoff (Sharpley et al., 2003).

As P-based management has advanced, there has been a growing interest in managing applied P sources (e.g., mineral fertilizers, manures, and biosolids) on the basis of their availability to runoff. Moore et al. (2000) reported significant differences in dissolved P losses from pastures with surface-applied chicken (*Gallus gallus domesticus* L.) litter, depending on whether or not it was treated with aluminum sulfate (alum). They observed concurrent declines in the water-extractable P (WEP) content of alum-treated litter relative to untreated litter and dissolved P concentrations in runoff from pastures receiving alum-treated litter compared with the untreated litter. Treatment of dry litters with alum is a recommended practice for reducing dissolved P losses from manure-amended soils (Moore, 2005). Inherent differences in manures from different livestock species can also result in different potentials for P loss in runoff. Kleinman et al. (2002b) observed significant differences in dissolved P concentrations in runoff from packed soil boxes receiving surface applications of different livestock manures (dairy cattle [*Bos taurus* L.], chicken, and swine [*Sus scrofa domestica* L.]) at an equivalent rate of total P addition. In the Moore et al. (2000) and Kleinman et al. (2002b) studies, dissolved P from surface-applied manures served as the primary source (>60%) of total P in runoff. Both studies found correlations between the WEP concentration of applied manure and dissolved P concentrations in runoff. More recent studies confirm that WEP is an effective quantitative indicator of dissolved P loss in runoff from surface applied manures and biosolids (Brandt and Elliott, 2003; Kleinman and Sharpley, 2003; Kleinman et al., 2004; Maguire et al., 2005).

There is no single recommended protocol for measuring WEP in manures and biosolids. The first published WEP protocols—Self-Davis and Moore (2000) and Sharpley and Moyer (2000)—differed in key areas. The Self-Davis and Moore method was developed for chicken litters, requiring a 20-g sample (wet weight) to minimize sampling error associated with heterogeneous litters, which is shaken for 2 h in 200 mL water. The Sharpley and Moyer method was originally used to determine WEP in manures with solids ranging from 11 to 89%, extracting 1 g (dry weight equivalent) of fresh manure with 200 mL water for 1 h. Although both methods analyze fresh manure, the Self-Davis and Moore method calls for a wet weight measurement of manure, whereas the Sharpley-Moyer method uses a ratio based on dry weight equivalency. Later work by Kleinman et al. (2002b) revealed that both methods were quite precise when tested on chicken, dairy, and swine manures with solids contents ranging from 1.6 to 52.7%. Replicated extractions produced similar CVs for the two methods, ranging from 0.01 to 0.12.

Although wet-weight determination involves less time and laboratory resources than predetermination of manure solids content to fix the extraction ratio, wet-weight determinations allow for considerable variability in the extraction ratio (solution:solids) of different manures. Because fresh manures contain some water, extracting solution contents include the deionized water added to

the manure and the water already in the manure. In the study by Kleinman et al. (2002b), extraction ratios (solution:solids) under the Self-Davis and Moore method ranged from 20:1 to 687:1 for the manures included in that study, whereas for the Sharpley and Moyer method the range was 201:1 to 262:1. When Kleinman et al. (2002b) conducted a runoff experiment with the same manures used to test the methods of Self-Davis and Moore and Sharpley and Moyer, the wide range of extraction ratios for the Self-Davis and Moore method led to a much lower coefficient of determination between dissolved P concentration in runoff and WEP of surface-applied manures ( $r^2 = 0.65$ ) than did the Sharpley and Moyer method ( $r^2 = 0.97$ ). Although the Self-Davis and Moore method was not intended to be a universal extraction method, the findings of Kleinman et al. (2002b) point to the need to maintain a fixed extraction ratio when comparing WEP concentrations across different types of manures. Recent research by Haggard et al. (2005) confirms the importance of controlling extraction ratios in using manure WEP to predict runoff P losses.

Although much of the original application and evaluation of WEP methods was derived from a research context, application of WEP has extended outside of the academic realm to nutrient management policies. Increasingly, P site assessment indices and fate-and-transport models are turning to WEP as a means of representing P in applied manures and biosolids that is at greatest risk to loss in runoff. For instance, the Arkansas P Index for Pastures, which was designed to improve management of surface-applied poultry litters to pastures, uses only WEP in applied poultry litter in estimating site vulnerability to P loss (Delaune et al., 2004). Coale et al. (2004) describe a slightly different use of WEP in P Indices of mid-Atlantic states in the USA, where a variety of manures and biosolids are applied to pastures and a variety of other agricultural systems. Mid-Atlantic P Indices use the WEP of applied manures and biosolids in the calculation of P source coefficients that represent the relative potential of an applied P source to release dissolved P to runoff (as opposed to particulate, or total P, in runoff). Material-specific P source coefficients will some day be derived by submitting manure or biosolids to a laboratory for WEP analysis. Thus, commercial testing laboratories will be expected to offer WEP as an environmental manure test.

Given growing interest in WEP testing, this study sought to determine whether a single WEP test for manures and biosolids could be recommended in an effort to standardize testing protocols and improve comparability of commercial WEP testing data. Building on previous research, the study first examines inter- and intralaboratory effects of methodologic factors on WEP extraction of 20 manures and biosolids. Subobjectives of this multilaboratory component were to quantify (i) interlaboratory variability in WEP methods for a single reference sample (multilaboratory experiment A), (ii) intralaboratory variability in WEP associated with extraction ratios on 20 different manures and biosolids (multilaboratory experiment B), and (iii) sample weight effects on WEP recovery and intralaboratory variability (multilaboratory experiment C). A subset of the manures and biosolids were used in a single laboratory WEP extraction and runoff study (experiment D) to assess the

Table 1. General properties of manures or biosolids used in multilaboratory experiments (A, B, C) and runoff experiment (D).

Sample ID	Material type	Description	Solids	Additives/treatment	Location	Experiment
			%			
Beef-1	beef feedlot manure	stockpile	79		Stillwater, OK	B, C, D
Biosolid-1	biosolids	municipal WWTP†	18		Altoona, PA	B, C, D
Biosolid-2	biosolids	municipal WWTP	16	alum	Bellefonte, PA	B, C, D
Biosolid-3	biosolids	municipal WWTP	13	ferric chloride	University Park, PA	B, C, D
Biosolid-4	biosolids	municipal WWTP	27	lime, ferric chloride	Washington, DC	B, C, D
Biosolid-5	biosolids	municipal WWTP	23	ferric chloride	Baltimore, MD	B, C
Biosolid-6	biosolids	municipal WWTP	41	biological P removal, lime	Savage, MD	B, C
Biosolid-7	biosolids	municipal WWTP	20		Hillsboro, OR	D
Biosolid-8	biosolids	municipal WWTP	23		Hillsboro, OR	B, C
Chicken-1	chicken litter	stockpiled litter	82		Raleigh, NC	A
Chicken-2	chicken litter	flock, decake, yearly cleanout	72		Wesley, AR	B, C, D
Chicken-3	chicken litter	flock, decake, yearly cleanout	72	alum	Wesley, AR	B, C, D
Chicken-4	chicken manure	under slatted, caged floor	40		Raleigh, NC	B, C
Dairy-1	dairy manure	bedded pack	18		State College, PA	B, C, D
Dairy-2	dairy manure	daily scrape	14		Kennett Square, PA	B, C, D
Dairy-3	dairy manure	scraped and composted	60		Twin Falls, ID	B, C, D
Dairy-4	dairy manure	scraped	19		Kingsville, Ontario, Canada	B, C, D
Dairy-5	dairy manure	daily scrape	14		Cochranville, PA	B, C
Swine-1	swine slurry	slurry tank	3		University Park, PA	B, C, D
Turkey-1	turkey litter	stockpiled litter	55		Ellsworth, IA	B, C, D

† WWTP, waste water treatment plant.

effect of methodologic differences in WEP tests on the prediction of runoff P losses from soils broadcast with manure.

## Materials and Methods

### Multilaboratory Extraction Study

A cooperative study comparing key elements of WEP protocols was conducted by 10 laboratories in the USA and Canada. Laboratories included Agri-Food Canada Environmental Health Laboratory (Harrow, ON); North Carolina State University (Raleigh, NC); Oklahoma State University Soil, Water and Forage Analytical Laboratory (Stillwater, OK); Oregon State University Central Analytical Laboratory (Corvallis, OR); University of Delaware (Newark, DE); University of Pennsylvania (Kennett Square, PA); USDA-ARS National Soil Tilth Laboratory (Ames, IA); USDA-ARS Northwest Irrigation and Soils Research Laboratory (Kimberly, ID); USDA-ARS Pasture Systems and Watershed Management Research Unit (University Park, PA); and USDA-ARS Poultry Production and Product Safety Research Unit (Fayetteville, AR).

#### Reference Sample Comparison (Multilaboratory Experiment A)

Interlaboratory experiments were conducted on a single reference sample at three extraction ratios (10:1, 100:1, and 200:1) to evaluate variability among laboratories performing WEP. The reference sample, a chicken litter containing wood chips up to 1 cm in diameter, was collected from a commercial farm in Raleigh, NC. The litter was mixed thoroughly and divided into 300-g subsamples for distribution among participating laboratories. Litter samples were shipped (overnight) on 14 Feb. 2005 to cooperating laboratories. Upon receipt, the reference litter sample was immediately refrigerated (4°C) and held in storage for a maximum of 20 d before analysis. Laboratories

were instructed to bring the litter to room temperature (approximately 25°C) and to conduct WEP extractions in triplicate at extraction ratios of 10:1, 100:1, and 200:1 as follows:

- (1) Determine the solids content of the litter by the method of Peters (2003).
- (2) Weigh, in triplicate, samples (as-received basis) containing 0.5 g (200:1 ratio), 1.0 g (100:1 ratio), and 10 g (10:1 ratio) of solids into appropriately sized flasks.
- (3) Add deionized water to achieve final extraction ratios (dry weight equivalent) of 200:1 (100.5 g total), 100:1 (101.0 g total), and 10:1 (110.0 g total).
- (4) Agitate samples for 1 h on an orbital shaker at 150 rpm, on a reciprocal shaker at 120 epm, or on an end-over-end shaker at 12 rpm.
- (5) Centrifuge (10 min at 1500 × g) and decant sample; if particulates are observed in the supernatant, filter sample (Whatman #40).
- (6) Measure P in the filtered or centrifuged extract by inductively coupled plasma (ICP) (measured by six laboratories) or colorimetry (measured by six laboratories), with two of the cooperating laboratories measuring P by both methods.

#### Extraction Ratio Effects across Diverse Manures and Biosolids (Multilaboratory Experiment B)

The 10 cooperating laboratories analyzed one or more manure or biosolids samples obtained from local sources to test for the effects of extraction method. Samples were selected to represent locally important manures and biosolids and included one swine manure, one beef cattle manure, five dairy cattle

**Table 2. Chemical properties of manures and biosolids used in the runoff study (experiment D).**

Material	Total P	Total N	Total N/total P	pH
	—g kg <sup>-1</sup> †			
Beef-1	6.9	23.4	3.4	7.9
Biosolid-1	18.2	41.8	2.3	6.9
Biosolid-2	24.5	56.6	2.3	6.6
Biosolid-3	40.4	76.5	1.9	7.6
Biosolid-4	16.5	50.1	3.0	12.1
Biosolid-7	29.4	63.0	2.1	8.5
Chicken-1	12.7	27.7	2.2	8.6
Chicken-2	18.6	39.5	2.1	8.6
Chicken-3	13.6	50.8	3.7	7.7
Dairy-1	5.6	26.5	4.7	8.4
Dairy-2	5.6	33.3	5.9	7.1
Dairy-3	3.1	11.4	3.7	7.3
Dairy-4	3.8	25.4	6.7	9.3
Swine-1	39.4	107.2	2.7	7.0
Turkey-1	17.0	46.1	2.7	6.7

† Dry weight equivalent.

manures, five poultry manures (three broiler litters, one broiler breeder, and one turkey litter), and eight biosolids (Table 1). As with the reference sample, these samples were mixed thoroughly and refrigerated at 4°C for a maximum of 20 d before analysis. Cooperators analyzed WEP in the sample(s) they collected at extraction ratios of 10:1, 100:1, and 200:1, following the identical extraction, filtering, and P-determination protocols used with the interlaboratory reference sample.

#### Sample Weight Effects on Variability of Water-Extractable Phosphorus of Diverse Manures and Biosolids (Multilaboratory Experiment C)

The 200:1 extraction ratio method, as developed by Wolf et al. (2005), calls for a 0.5-g sample (dry weight equivalent basis) and a final solution volume (water in manure plus water added) of 100 mL. There exist concerns about the small amount of sample used in this protocol, particularly for heterogeneous manures such as poultry litters. In contrast, the 10:1 extraction ratio method can use a much larger amount of sample (approximately 10 g solids) for each 100 mL of solution. The objective of this experiment was to determine the importance of sample weight on laboratory precision at the 10:1 and 200:1 extraction ratios and whether increasing the amount of a sample used in WEP analysis improves laboratory precision.

Each laboratory brought the material it had collected (Table 1) to room temperature and measured WEP in “as is” material at extraction ratios of 10:1 and 200:1 following the steps described for the interlaboratory experiment but with several key differences. To ensure a sufficient number of observations for accurate measurement of variability in WEP, each treatment was replicated eight times. With the 10:1 extraction ratio, 10, 20, and 100 g of solids were used. With the 200:1 extraction ratio, 0.5, 1.0, and 5 g of solids were used.

#### Single Laboratory Water-Extractable Phosphorus Extractions and Runoff Study (Experiment D)

A series of WEP extractions was performed by the USDA-

ARS Pasture Systems and Watershed Management Research Unit (University Park, PA) to determine whether individual components of the WEP protocol affected the correlation with runoff P. To facilitate the discussion of experimental results, we have divided this experiment into two sub-experiments: D1 and D2. Experiment D1 consists of laboratory WEP extractions of 15 manures and biosolids. Experiment D2 is a rain simulation study quantifying dissolved P release to runoff from the 15 manures and biosolids used in experiment D1.

Participating laboratories shipped at least one, well mixed sample of manure or biosolids for experiment D (Table 2). All but one of these samples (Biosolid-7) were included in the multilaboratory experiments (A, B, and C). Samples were shipped via express delivery in insulated containers with ice packs to minimize microbial activity and associated P transformations. Upon receipt, samples were immediately refrigerated (4°C), with a maximum storage time of 14 d before performing laboratory analyses and the runoff study.

As with the multilaboratory extraction experiments A, B, and C, the WEP of manures and biosolids was determined at 10:1, 100:1, and 200:1 extraction ratios in experiment D1. To test for the effect of solid separation procedure on WEP determination, three methods were used: centrifugation only, centrifugation followed by filtration with Whatman #1 filter, and centrifugation followed by filtration with Whatman #40 filter. Filtrates were analyzed for phosphorus determined by colorimetry ( $P_{\text{color}}$ ) by a modified Murphy and Riley (1962) method ( $\lambda = 712 \text{ nm}$ ) and for phosphorus determined by inductively coupled plasma–atomic emission spectrometry ( $P_{\text{ICP}}$ ) by ICP at Penn State’s Agricultural Analytical Services Laboratory. All extractions were conducted in triplicate. In addition to WEP determination, all manures and biosolids were submitted to Penn State’s Agricultural Analytical Services Laboratory for routine testing. These additional tests included total P (USEPA Method 3051 using microwave digestion), total N (combustion analysis with an Elementar Vario Max CN Analyzer), solids content (gravimetric determination after 14-h drying at 105°C), and pH (2:1, water:manure).

For the runoff study (experiment D2), manures and biosolids were applied to packed soil boxes at a constant rate of total phosphorus (TP) addition at the USDA-ARS Pasture Systems and Watershed Management Research Unit (University Park, PA). Phosphorus in collected runoff from the soil boxes was determined by ICP at the Penn State Agricultural Analytical Services Laboratory. The runoff study was conducted following the National P Research Project packed box protocol (National Phosphorus Research Project, 2005). The surface horizon (0–20 cm) of a Hagerstown soil (fine, mixed, semiactive, mesic Typic Hapludalf) was collected from a field on Pennsylvania State University’s Larson Agronomy Farm (Rock Springs, PA). The field was in long-term corn-soybean-small grain rotation with a history of receiving dairy manure in excess of crop P requirement. The soil was field sieved (1.4 cm), air-dried, and thoroughly mixed. The mixed soil was analyzed for Mehlich-3 P (Mehlich, 1984) by shaking 2.5 g of soil with 25 mL of Mehlich-3 solution ( $0.2 \text{ M CH}_3\text{COOH} + 0.25 \text{ M NH}_4\text{NO}_3 + 0.015 \text{ M NH}_4\text{F} + 0.013 \text{ M HNO}_3 + 0.001 \text{ M EDTA}$ ) for 5 min. The supernatant



was filtered (Whatman #1), and P was determined colorimetrically in the neutralized filtrate. To ensure homogeneity of the mixed soil, the effectiveness of mixing was evaluated by conducting Mehlich-3 P extraction on six subsamples and determining the CV (SD divided by mean Mehlich-3 P concentration). The mean Mehlich-3 P concentration was 98 mg kg<sup>-1</sup>, and the CV was less than 0.04. The mean pH of the soil (soil:water = 1:1) was 6.4. The soil had a texture of clay loam (27% sand, 42% silt, 31% clay).

The Hagerstown soil was packed into stainless steel boxes to achieve an approximate bulk density of 1.3 to 1.5 g cm<sup>-3</sup>. Boxes were 1 m long, 20 cm wide, and 5 cm deep, with back walls 2.5 cm higher than the soil surface and 5-mm-diameter drainage holes in the base. Cheesecloth was placed on the bottom of each box before packing the box with soil. At the lower end of each box, a gutter equipped with a canopy channeled runoff water to collection containers (Kleinman et al., 2002a). Packed soil boxes were placed under a rainfall simulator described by Humphry et al. (2002) equipped with a TeeJet 1/2 HH SS 30 WSQ nozzle (Spraying Systems Co., Wheaton, IL). The nozzle, located approximately 3 m above the packed boxes, produced simulated rainfall that achieved greater than 90% terminal velocity and had a coefficient of uniformity of greater than 0.85 within the area where the soil boxes were placed. Filtered tap water (dissolved P<sub>color</sub> <0.005 mg L<sup>-1</sup>; electrical conductivity = 0.019 dS m<sup>-1</sup>) served as the source of rainfall and was applied at an intensity of 6.0 cm h<sup>-1</sup>.

On 22 Feb. 2005, soils were saturated using the rainfall simulator and allowed to drain for 72 h before application of manures and biosolids. At the time of application, all packed soils were approximately at field capacity ( $\theta \sim 0.30$ , confirmed by capacitance sensor), ensuring that hydrologic variability related to antecedent moisture was minimized. On 25 Feb. 2005, manures and biosolids were broadcast onto boxes at a rate of total P addition equivalent to 75 kg ha<sup>-1</sup>. Kleinman and Sharpley (2003) determined that a manure application rate of at least 50 kg TP ha<sup>-1</sup> is required to consistently detect differences in runoff dissolved P as a function of WEP of applied manures. At time of application, all boxes were placed on a horizontal surface so that there was no preferential accumulation of applied solids or water at the lower end of the box. Furthermore, to ensure that the swine slurry was evenly distributed within the soil box and did not run off during the application process, a 7.5-cm-high plastic collar was temporarily placed inside the walls of the box. After the swine slurry water had infiltrated into the horizontally seated box, the collar was removed, leaving the slurry uniformly distributed across the surface of the soil box. A set of unamended soil boxes was included as a control. All treatments were performed in triplicate.

Rainfall simulations were conducted on 28 Feb. 2005, approximately 72 h after manures and biosolids were applied to the soil boxes. Runoff boxes were placed under the rain simulator, inclined to a 3% slope gradient, and staggered so that during rainfall simulation splash from one box would not be intercepted by another box. Rainfall simulations persisted until 30 min of runoff was collected from each of the soil boxes. After rainfall simulation, a single, composite sample was collected for the entire 30-min runoff event and immediately filtered (0.45

**Table 3.** Effect of extraction ratio and phosphorus determination method on water-extractable phosphorus (WEP) and relative standard deviation (RSD) across laboratories for the Chicken-1 reference sample (multilaboratory experiment A).

Extraction ratio	N†	ICP-AES‡		Colorimetric	
		WEP	RSD	WEP	RSD
mL/g		g kg <sup>-1</sup> §	%	g kg <sup>-1</sup>	%
10:1	6	0.6	7.6	0.5	20.7
100:1	6	1.6	11	1.4	9.6
200:1	6	1.8	7.7	1.6	8.5

† Number of laboratories.

‡ Inductively couple plasma–atomic emission spectrometry.

§ Dry weight equivalent.

µm). Filtered runoff samples were analyzed for P<sub>color</sub> and P<sub>ICP</sub> within 24 h of collection as described previously.

## Data Analysis

For the multilaboratory extraction experiments (A, B, and C), WEP data from each of the cooperating laboratories were compiled in a common database. Variability in WEP measurement within and between laboratories was described by relative standard deviation (RSD). Statistical comparison of means was conducted by ANOVA, with mean categories identified using Tukey's test.

For the single-laboratory extraction experiment and runoff study (D1 and D2), differences in WEP of applied P sources and dissolved P in runoff were assessed by Student's *t* test or by ANOVA. Relationships between runoff P and WEP were assessed by Pearson's correlation analysis and modeled by least squares linear regression. Statistical results discussed below are significant at  $\alpha = 0.05$ .

## Results and Discussion

### Multilaboratory Extraction Study

#### Reference Sample Comparison (Multilaboratory Experiment A)

Relative SDs in WEP of the reference manure (Chicken-1) ranged from 7.6 to 20.7% (Table 3), consistent with variability in other manure analyses (e.g., solids content, total P) and within the range of RSDs reported by Wolf et al. (2005) for WEP in chicken (broiler and layer), dairy, and swine manures analyzed by eight different laboratories. Differences in RSDs were evident by the P determination method (ICP vs. colorimetric) and by extraction ratio.

Determination of P in water extracts by ICP produced relatively consistent WEP measurements, with RSDs ranging from 7.6 to 11.0 and no distinct trend across extraction ratios. These RSDs are lower than the RSD of 13.3 reported by Wolf et al. (2005) for a chicken litter sample and may in part reflect greater homogeneity in the litter analyzed in the current study. Determination of P by colorimetry produced a greater range of RSDs (Table 3). At extraction ratios of 100:1 and 200:1, RSDs for colorimetry were comparable with those found with ICP. A much higher RSD (20.7) was observed at the 10:1 extraction ratio with colorimetry. At the 10:1 ratio, the water extracts of the litter were relatively dark in color and more turbid than extracts from the 100:1 and 200:1 extraction ratios, where greater amounts of deionized water helped to dilute the color. To avoid interference, some of the laboratories diluted

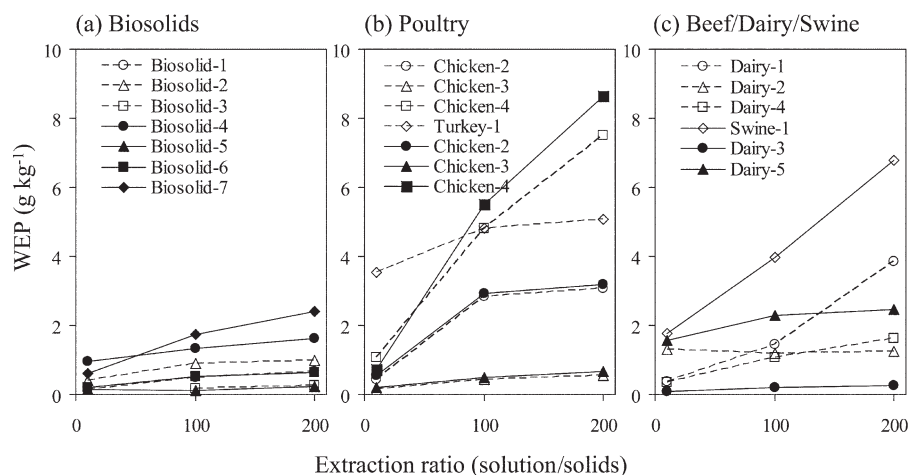


Fig. 1. Effect of extraction ratio (10:1, 100:1, 200:1) on water-extractable phosphorus (WEP) of manures and biosolids measured by 10 laboratories in experiment B. Open symbols indicate colorimetric determination of WEP. Closed symbols indicate inductively coupled plasma-atomic emission spectrometry determination of WEP.

the extract as part of the colorimetric measurement procedure. Thus, the higher variability of the 10:1 extract for the colorimetric procedure can likely be attributed to the difficulties of performing this measurement on darkly colored and turbid samples and to the different approaches taken by laboratories in its measurement.

#### Extraction Ratio Effects across Diverse Manures and Biosolids (Multilaboratory Experiment B)

Laboratories determined WEP in a total of 20 manures and biosolids (Table 1) at extraction ratios of 10:1, 100:1, and 200:1. Extraction ratio and WEP were positively correlated for all samples (Fig. 1), increasing an average of 2.0-fold (range, 0.9–7.6) from

Table 4. Extraction ratio and sample weight effects on within-laboratory variability in water-extractable phosphorus (WEP) (multilaboratory experiment C).

Extraction ratio	Sample weight (dry weight equivalent)	RSD†	
		WEP-colorimetric	WEP-ICP-AES‡
		%	
mL/g	g		
N = 8			
10:1	10	19.7 (28.9)	
10:1	20	10.0 (9.3)	
10:1	100	7.2 (3.9)	
N = 10			
10:1	10		11.7 (10.2)
10:1	20		12.1 (9.2)
10:1	100		12.0 (12.1)
N = 12			
200:1	0.5	7.2 (4.5)	
200:1	1	8.9 (2.5)	
200:1	5	7.2 (4.2)	
N = 10			
200:1	0.5		7.3 (5.2)
200:1	1		8.1 (7.1)
200:1	5		8.9 (7.6)

† Mean relative standard deviations (RSD) of laboratories specified (N) based on eight replications per laboratory. Value in parentheses is SD.

‡ Inductively coupled plasma-atomic emission spectrometry.

10:1 to 100:1 and an average of 1.4-fold (range, 1.1–2.6) from 100:1 to 200:1. Kleinman et al. (2002b) noted similar increases in WEP of poultry, swine, and dairy manures with extraction ratio and attributed this relationship to the greater dissolution of phosphate compounds (e.g., calcium phosphates) at the higher extraction ratios. In the current study, relationships between WEP and extraction ratio are clearly specific to individual materials, with no consistent generalizations evident when materials are clustered into different categories (Fig. 1). The slope of the WEP-extraction coefficient relationship likely depends on a variety of factors, including the concentration of P-reactive compounds in the material, the relative affinity of such compounds to P, the proportion of total P that is water extractable at lower extraction ratios, and the solids content of the material.

In addition to differences in P recovery, varying the extraction ratio was accompanied by certain logistic problems for the broad range of materials evaluated in this study. The 10:1 ratio was initially developed for use on poultry manures (Self-Davis and Moore, 2000), and several cooperators noted difficulties when using this ratio in extracting other materials, particularly dairy manures and some biosolids. Experimental difficulties and concerns noted with the 10:1 ratio included (i) shaking/mixing problems because the sample would adhere to the extraction vessel rather than flow freely, (ii) challenges in obtaining sufficient extract for ICP or colorimetric analysis, (iii) concerns with clogging of ICP tubing because of high particulate concentration, and (iv) concerns with color interference when performing colorimetric analysis. Although several of these issues (clogging of ICP tubing and color interference) were raised in regard to the other extraction ratios, they were most problematic with the 10:1 ratio. When performing the 10:1 ratio, several cooperators took extra time or performed additional steps (such as diluting the extract, filtering through a 0.45- $\mu$ m filter, or centrifuging at a speed higher than that recommended in the protocol) to obtain sufficient extract for analysis. One biosolids sample (Biosolid-3) could not be extracted at 10:1 because no supernatant could be obtained.

#### Sample Weight Effects across Manures and Biosolids (Multilaboratory Experiment C)

Varying the weight of sample used in the extraction procedure did not significantly affect intralaboratory error associated with the method (Table 4). With the exception of the mean RSD for the colorimetric procedure performed on the 10:1 extraction ratio with a sample size of 10 g (RSD, 19.7), the mean intralaboratory RSDs related to sample weight for all other treatments ranged from 7.2 to 12.1 (Table 4). Although the mean intralaboratory RSD values were close to each other when averaged over all materials, there was considerable variability in RSDs as revealed by the high SDs (Table 4).

The highest intralaboratory RSDs arose with larger samples. The highest RSD was associated with a dairy manure (Dairy-4) at the 10:1 ratio from a 10-g sample with extract P determined by colorimetry (hence the large deviation in RSDs for this protocol). Colorimetric measurements on dairy manures were particularly problematic at the 10:1 extraction ratio. Differences in WEP related to sample weight were not related to material type (poultry, dairy, swine, beef manure, or biosolids), P determination method (colorimetric, ICP), or extraction ratio (10:1, 200:1). Approximately 75% of the samples in which sample weight did affect WEP concentration followed the trend of WEP decreasing with increasing sample weight. For these samples, the average increase in WEP from the highest to the lowest sample weight was 1.7-fold (range, 1.1- to 5.3-fold). The remaining samples showed no trend with sample weight or a showed trend of WEP increasing with increasing sample weight.

Variability in the effect of sample weight on WEP points to a variety of processes potentially affecting WEP measurement. Differences in precision of WEP measurement with sample weight may in part be related to subsampling bias. When sampling a 0.5-g sample size for the 200:1 ratio on heterogeneous materials, there may be a tendency to sample smaller particle sizes (avoiding large particles) rather than a range of sizes that could be subsampled when taking a larger (10-g) sample. Because P is unequally distributed among particles of different sizes (e.g., fine feces particles vs. coarse wood chips), greater variability could result when a broad range of particles was sampled (e.g., with a 10-g subsample) than with a select subset (e.g., with a 0.5-g subsample). Indeed, P recovery varied significantly with sample weight for 67% of the manures and biosolids tested in this study, suggesting bias related to sample weight.

Sampling bias alone cannot explain the differences because materials that were heterogeneous (e.g., litters, bedded pack) showed no greater tendency to the phenomenon than did materials that were homogeneous (e.g., biosolids, composts). The type of extraction vessel (size and shape) and head space likely influenced the recovery of P. The importance of headspace in an extraction process has been noted by others (e.g., Eliason, 1998). The experimental protocol, designed to reflect variability in basic laboratory practices, equipment, and labware, did not control for head space. Cooperators used shakers and labware available at each laboratory, resulting in ratios of solution to extraction vessel volume that ranged from 0.2 to 1.0. There was no consistent relationship between head space available and WEP extracted for those manures and biosolids where sample weight affected WEP recovery.

### Single-Laboratory WEP Extractions and Runoff Study (Experiment D)

Results of the runoff study confirm the potential for recently applied P sources to enrich dissolved P in runoff (Table 5). As observed in previous studies investigating the effects of surface P application on runoff (e.g., Edwards and Daniel, 1993; Moore et al., 2000), the lowest dissolved P concentrations in runoff were from the control soils that did not receive P amendments. Concentrations of dissolved P in runoff from amended soils varied

widely (0.18–29.96 mg L<sup>-1</sup>), with means ranging from biosolids with the lowest dissolved P concentrations of any category to turkey litter with the highest dissolved P concentrations. Trends in dissolved P loss (Table 5) here are consistent with those reported elsewhere. For instance, differences in runoff P concentrations between chicken litter treatments confirm the benefit of alum treatment in reducing dissolved P losses in runoff, such as described by Moore et al. (2000). As in the current study, Kleinman and Sharpley (2003) observed that dissolved P concentrations in runoff were higher from surface-applied swine and chicken manures than from surface-applied dairy manure. Similarly, Brandt and Elliott (2003) reported that runoff from surface-applied biosolids contained significantly lower dissolved P concentrations than runoff from chicken manure.

Trends in the dissolved P concentration of runoff were similar for colorimetric and ICP methods of P determination in runoff water (Table 5), reflecting the strong correlation between the two methods ( $r = 0.99$ ; runoff dissolved  $P_{ICP} = 1.18$  runoff dissolved  $P_{color} + 0.25$ ). As suggested by the regression describing this relationship, dissolved  $P_{ICP}$  was significantly greater than dissolved  $P_{color}$  in runoff water. Various authors (e.g., Brandt and Elliott, 2003; Pittman et al., 2005) have likened  $P_{ICP}$  with total P in solution, whereas  $P_{color}$  has been equated with dissolved inorganic P. Differences between  $P_{color}$  and  $P_{ICP}$  are often attributed to organic and colloidal forms of P in solution that are not measured by colorimetry (Kuo, 1996; Mallarino, 2003), although some authors have called such an assumption into question (e.g., Turner et al., 2004). Given the significant differences in  $P_{ICP}$  and  $P_{color}$  of the filtered runoff water, it is likely that organic and colloidal forms of P contributed to the systematic difference observed between  $P_{color}$  and  $P_{ICP}$  in runoff from the current study. This inference is supported by the growing body of evidence that organic P is a significant component of P in solutions from manure and manured soils (Dou et al., 2002; He et al., 2004; Maguire et al., 2004) and by the observation that large amounts of manure and soil solids erode under runoff experiments of this type (Kleinman et al., 2004).

### Influence of Water-Extractable Phosphorus Extraction Protocol on

Table 5. Concentration of dissolved P in runoff from packed soil boxes receiving surface applications of manure or biosolids (experiment D2).

Treatment	N	Colorimetric determination of dissolved P		ICP-AES determination of dissolved P†	
		Mean	SD	Mean	SD
		mg L <sup>-1</sup>			
Control	1	0.18	–	0.21	–
Biosolids	5	1.19	0.88	1.72	1.08
Cattle manures					
Beef	1	9.41	–	11.87	–
Dairy	3	5.99	4.19	7.32	4.57
Broiler chicken litter					
Untreated	2	9.11	1.87	10.78	2.27
Alum treated	1	3.22	–	3.95	–
Swine slurry	1	10.05	–	11.42	–
Turkey litter	1	22.25	–	27.05	–

† Inductively coupled plasma–atomic emission spectrometry.

Table 6. Effect of water-extractable phosphorus (WEP) filtration method, WEP determination method (inductively coupled plasma–atomic emission spectrometry [ICP–AES] or colorimetric), and runoff P determination method (ICP–AES or colorimetric) on correlation between manure or biosolids WEP (mg kg<sup>-1</sup>) and dissolved P concentration in runoff (mg L<sup>-1</sup>) (experiment D).

Filtration	WEP determination	Runoff P determination method					
		WEP extraction ratio = 10:1		WEP extraction ratio = 100:1		WEP extraction ratio = 200:1	
		Colorimetric	ICP–AES	Colorimetric	ICP–AES	Colorimetric	ICP–AES
Centrifuge only	colorimetric	0.90†	0.90	0.92	0.93	0.88	0.87
Centrifuge only	ICP–AES	0.90	0.90	0.91	0.92	0.85	0.86
Whatman #1	colorimetric	0.90	0.91	0.90	0.91	0.84	0.83
Whatman #1	ICP–AES	0.91	0.91	0.89	0.90	0.84	0.84
Whatman #40	colorimetric	0.90	0.91	0.90	0.91	0.81	0.79
Whatman #40	ICP–AES	0.91	0.92	0.89	0.90	0.85	0.85

† All *p* values < 0.001

### Correlation with Runoff Phosphorus

Dissolved P concentration of runoff was strongly correlated with the concentration of WEP of applied P sources expressed on a dry-weight–equivalent basis (Table 6), confirming the potential for WEP to serve as an indicator of dissolved P loss across a wide range of P sources. All WEP protocols tested by this study produced *r* values ≥ 0.79 (Table 6), although correlations reported for the 10:1 ratio do not include all samples due to difficulties in the extraction process described previously.

Effect of Water-Extractable Phosphorus Extraction Ratio (10:1, 100:1, 200:1) on Correlation with Runoff Phosphorus. Figure 2 illustrates differences in the relationship between dissolved P in runoff and WEP of applied P sources as affected by extraction ratio. Data in this figure are for only one of the methodologies tested in experiment D (centrifugation only, colorimetric determination of P in runoff and in water extracts) but are representative of trends observed with the other WEP methods evaluated in experiment D. The steepest regression slopes between dissolved P in runoff and WEP were observed at the 10:1 ratio (Fig. 2a), reflecting the lesser quantities of WEP recovered with this ratio. Although fewer samples were analyzed at the 10:1 ratio, those samples that were analyzed represented the complete range of WEP concentrations measured

by the 100:1 and 200:1 protocols such that the smaller data set should not have skewed the 10:1 findings. Observations at the 10:1 ratio are relatively tightly clustered, indicating, in part, lesser resolution in WEP. A key attribute of a quantitative indicator is to provide consistent distinction of potential (in this case potential for dissolved P release to runoff) across the range of observations in the sample universe (Neter et al., 1996). Both 100:1 (Fig. 2b) and 200:1 (Fig. 2c) extraction ratios produce broader spreads of WEP, with 100:1 exhibiting the most even distribution of observations across the range of biosolids and manures included in this study. Correlations obtained from WEP with a 100:1 extraction ratio were consistently significantly higher than those obtained from the 200:1 extraction ratio (Table 6).

Effect of Phosphorus Determination Method on Correlation with Runoff Phosphorus. No statistically significant differences in the strength of the correlation between dissolved P in runoff and WEP were apparent as a function of P determination method (colorimetric or ICP) used in the WEP protocol (Table 6). If substantially different pools of P in solution were determined by ICP and colorimetric methods, a difference in the correlation with runoff P would be expected. Manure or biosolids WEP determined by colorimetric and ICP methods were highly

correlated ( $r = 0.99$ ;  $P_{\text{ICP}} = 1.12 P_{\text{color}} + 0.24$ ). The greater  $P_{\text{ICP}}$  values run counter to those of Wolf et al. (2005), who also compared P determination methods used in WEP testing. They found that  $P_{\text{color}}$  values tended to be larger than those of  $P_{\text{ICP}}$  and attributed the difference to color interference in dark, turbid manure water extracts. Wolf et al. (2005) tested a larger proportion of liquid manures, which tend to yield the darkest water extracts. However, the swine slurry tested in the current study did not conform to such a generalization, with  $P_{\text{color}}$  averaging 80% of  $P_{\text{ICP}}$  in water extracts from the swine slurry. These conflicting laboratory results temper generalizations regarding the effect of P determination methods on WEP, but runoff results clearly show

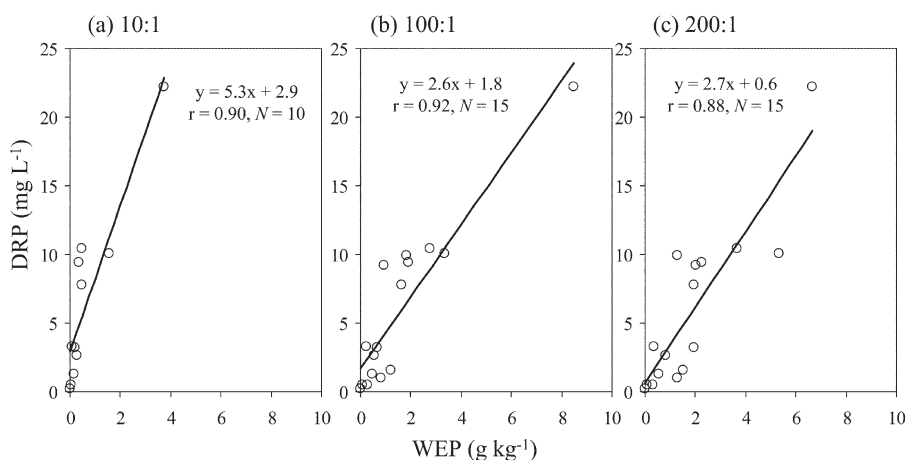


Fig. 2. Example of water-extractable P (WEP) extraction ratio effects on the relationship between manure or biosolids WEP and dissolved reactive P (DRP) concentration in runoff in experiment D. Phosphorus in runoff was determined by colorimetry. Only one set of WEP extraction protocols is presented (centrifugation, colorimetric P determination). The effects of additional variables on the correlation between biosolids or manure WEP and runoff P are reported in Table 6.



Table 7. Effect of water-extractable phosphorus (WEP) filtration method, WEP determination method (inductively coupled plasma–atomic emission spectrometry [ICP-AES] or colorimetric), and runoff P determination method (ICP-AES or colorimetric) on correlation between the fraction of total manure or biosolids P that is water-soluble (WEP/TP) and dissolved P concentration in runoff (mg L<sup>-1</sup>) (experiment D).

Filtration	WEP determination	Runoff P determination method					
		WEP extraction ratio = 10:1		WEP extraction ratio = 100:1		WEP extraction ratio = 200:1	
		Colorimetric	ICP-AES	Colorimetric	ICP-AES	Colorimetric	ICP-AES
Centrifuge only	colorimetric	0.91†	0.92	0.80	0.83	0.78	0.79
Centrifuge only	ICP-AES	0.92	0.93	0.79	0.82	0.72	0.75
Whatman #1	colorimetric	0.90	0.91	0.87	0.89	0.77	0.78
Whatman #1	ICP-AES	0.91	0.93	0.83	0.87	0.70	0.73
Whatman #40	colorimetric	0.89	0.91	0.88	0.90	0.77	0.78
Whatman #40	ICP-AES	0.91	0.92	0.83	0.86	0.73	0.76

† All *p* values < 0.001

that both methods of P determination are equally adequate in predicting dissolved P in runoff (Table 6).

#### Solid Separation Method Effects on Correlation with Runoff Phosphorus.

Across all of the extraction ratios and P determination methods evaluated in this study, solids separation method (centrifugation only, centrifugation + Whatman #1 filtration, centrifugation + Whatman #40 filtration) had no significant effect on the strength of the correlation between manure WEP and dissolved P in runoff (Table 6). Kleinman et al. (2002b), comparing Whatman #1 and 0.45- $\mu$ m filtration methods used in WEP analysis, found no effect of solid separation method on correlations between dissolved P in runoff and WEP of surface-applied manures. Similarly, Wolf et al. (2005) did not observe differences in P concentrations of water extracts obtained by centrifugation only or by Whatman #1 filtration.

**Water-Extractable Phosphorus Reporting Unit Effects on Correlation with Runoff Phosphorus.** Correlations were compared using different units for WEP: wet weight basis, WEP TP<sup>-1</sup> (Table 7), and dry weight basis (Table 6). When WEP results on a wet weight basis were compared with dissolved P concentrations in runoff, no significant correlations were observed (data not shown). When WEP was expressed as a fraction of total P in manure (WEP TP<sup>-1</sup>), as in Brandt and Elliott (2003), relationships with dissolved P in runoff were strong (Table 7), although not as consistently strong as those obtained by expressing WEP on a dry-weight–equivalent basis (Table 6), particularly at the 100:1 and 200:1 extraction ratios where all 14 manures and biosolids were included in the correlation analysis.

**Effect of Laboratory Conducting Water-Extractable Phosphorus Determination on Correlation with Runoff Phosphorus.** The same biosolids and manure samples were analyzed for WEP by many cooperating laboratories in multilaboratory experiments B and C and by a single laboratory (experiment D1). The rainfall simulation study clearly showed that WEP measured by a single laboratory (Experiment D1) was strongly correlated with dissolved reactive phosphorus concentration in runoff (experiment D2). To better understand the role of interlaboratory error on WEP as an indicator of P runoff potential, WEP results from experiments in the multilaboratory extraction study were related to runoff P results. Table 8 shows the correlations between WEP in manures and biosolids determined

in multilaboratory extraction experiments B and C versus dissolved P in runoff (experiment D2). Water-extractable P measurements are provided for each of the three extraction ratios tested in multilaboratory experiment B (10:1, 100:1, 200:1) and for varying sample weights tested in multilaboratory experiment C (10:1 and 200:1 only). Because laboratories determined P in water extracts by colorimetry or ICP, the effect of P determination method on WEP could not be controlled, so WEP values represented a combination of those determined by colorimetry and by ICP. Although correlations with data from the multiple laboratories (Table 8) were lower than those obtained from single laboratory (Table 6), they were nearly all significant and were consistent for runoff P determined by colorimetry and by ICP. Differences in correlations obtained from experiments B and C for 10:1 and 200:1 ratios using 10- and 0.5-g samples, respectively, reflect variability in means obtained from the two experiments despite identical protocols (Table 8). For 10:1 and 200:1 ratios, changing the sample weight substantially altered the strength of the correlation, with smaller sample weights producing stronger correlations with dissolved P in runoff.

These results highlight the potential for WEP measured by multiple laboratories to serve as an indicator of dissolved P

Table 8. Effect of water-extractable phosphorus (WEP) extraction ratio, sample weight used in WEP extraction, and runoff P determination method (inductively coupled plasma–atomic emission spectrometry [ICP-AES] or colorimetric) on the correlation between manure or biosolids WEP (mg kg<sup>-1</sup>) and dissolved P concentration in runoff (mg L<sup>-1</sup>). Manure or biosolids WEP measured in multilaboratory experiments B or C. Runoff P measured in experiment D.

Data source†	Extraction ratio	Sample weight	Runoff dissolved P	
			Colorimetric	ICP-AES
			<i>r</i>	
	mL/g	g		
B	10:1	10	0.71	0.74
B	100:1	1	0.86	0.86
B	200:1	0.5	0.76	0.75
C	10:1	10	0.69	0.73
C	10:1	20	0.65	0.68
C	10:1	100	0.56‡	0.57
C	200:1	0.5	0.81	0.81
C	200:1	1	0.62	0.61
C	200:1	5	0.61	0.60

† Letters indicate source of data as multilaboratory experiment B or C.

‡ Correlation not significant at  $\alpha = 0.05$ .

loss potential for land-applied manures and biosolids.

## Implications for Manure Testing

The selection of a testing protocol for use by commercial labs requires careful consideration of reproducibility, ease of implementation at cooperating laboratories, and adaptation to different manures and biosolids. In the case of WEP, an environmental indicator, the test must be well correlated with dissolved P in runoff. This section describes factors we considered in recommending a common WEP protocol.

The multilaboratory extraction study (experiments A, B, and C) produced several findings that influence the choice of an appropriate WEP test. A consistent trend in increasing WEP value with increasing extraction ratio (from 10:1 to 100:1 to 200:1) was observed for all 20 manures and biosolids tested in the study. However, there was considerable variability in the nature of this increase, with WEP increasing 0.9- to 7.6-fold (from 10:1 to 100:1) and 1.1- to 2.6-fold (from 100:1 to 200:1). Such variability does not support a simple proportional relationship between WEP values at different extraction ratios that would be useful for a wide range of P source types. To facilitate pooling of WEP data across experiments, it is critical that future research uses a common extraction ratio.

Cooperating laboratories were able to reproduce WEP values for the reference sample (experiment A) across all three extraction ratios with RSDs <20.7%, comparable to other, common manure and biosolids tests. When extracts obtained at a 10:1 ratio were analyzed by colorimetry, the RSD increased substantially because of interference related to dark, turbid extracts. Across all materials and extraction experiments, the 10:1 ratio was associated with a litany of difficulties not associated with the 100:1 and 200:1 ratios. Across all extraction ratios, clogging of ICP tubing and color interference were persistent concerns, but they were most problematic with the 10:1 ratio.

One concern about higher extraction ratios (100:1 and 200:1) reported by laboratory analysts was the need to use larger extraction vessels to accommodate the large volume of solution required of these ratios if a sample size larger than 0.1 to 1.0 g (dry weight equivalent) is used. Many laboratories in the current study and many commercial laboratories do not routinely use laboratory ware (extraction vessels) larger than 250 mL. Although this study shows that increasing sample weight does not improve the precision of the WEP test, systematic trends in WEP recovery with some materials (e.g., litters) point to potential for sampling bias. A WEP test should use sufficient sample to ensure that measured WEP values reflect manure and biosolids because they are land applied. Larger samples (e.g., 3–5 g) require larger extraction vessels (500 or 1000 mL). In addition to the inconvenience and costs associated with obtaining new labware and modifying existing equipment (e.g., orbital shakers) to accommodate the new labware, this study points to potential concerns with regard to variable headspace producing variable extraction efficiencies. Headspace concerns were most pronounced with the 1000-mL containers needed to maintain the 200:1 extraction ratio with larger manure and biosolids samples. However, 500 mL vessels

could be used for the 100:1 extraction ratio. Although 500-mL vessels are larger than the extraction vessels typically used by many laboratories, they are within a reasonable size to be readily incorporated into a commercial laboratory setting.

The runoff study (experiment D2) demonstrated that WEP from all extraction methods was well correlated with runoff dissolved P, even when WEP results were obtained from multiple laboratories (experiments B and C), although the best correlations were obtained when a single laboratory analyzed all samples (experiment D). Because the recovery of P with the 10:1 ratio was less than with the 100:1 or 200:1 ratios, the slope of the linear regression between dissolved P in runoff and WEP was steepest for the 10:1 ratio. Therefore, WEP developed at the 10:1 ratio had the lowest sensitivity in differentiating between sources of P to runoff. Of all ratios examined in this study, the highest correlations with dissolved P in runoff tended to be with the 100:1 ratio. Sample sizes are larger with 100:1 than 200:1 at the same extraction volume, which may be an advantage in some situations. One of the reasons the 10:1 ratio has traditionally been used for poultry litter analysis is the concern that some litters contain large pieces of bedding, making it difficult to obtain reproducible samples for extraction. Results of this study indicate that increasing sample weight does not improve the prediction of dissolved P in runoff from WEP.

Commercial laboratories measure P by different methods, making prescription of only ICP or only colorimetric P determination unrealistic. Cooperating laboratories in multilaboratory experiments were able to reproduce WEP values using ICP or colorimetry, although WEP determined by colorimetry was susceptible to interference-related error, particularly at the 10:1 extraction ratio. In the runoff study (experiment D), WEP from ICP or colorimetric P determination methods was strongly correlated to dissolved P in runoff. However, because ICP and colorimetric methods measure slightly different values for WEP (see also Wolf et al., 2005), caution is warranted in comparing WEP from laboratories that use different P determination procedures.

Results of this and similar studies (Kleinman et al., 2002b; Wolf et al., 2005) indicate that common solid separation methods used in testing laboratories do not significantly affect WEP values. Furthermore, it is unlikely that commercial labs would want to use 0.45- $\mu$ m or Whatman #40 filters due to difficulties in obtaining a filtrate. Thus, the recommended solid separation method of Wolf et al. (2005) would seem to be prudent: centrifugation only or centrifugation plus coarse filtration when necessary for P determination.

## Recommended Water-Extractable Phosphorus Protocol

Based on the findings of this study, we believe that a WEP test performed at the 100:1 extraction ratio offers a reasonable balance of precision and practicality demanded by commercial testing laboratories while serving as a consistent indicator of runoff P loss potential for a wide range of land-applied manures and biosolids. The 100:1 ratio had the highest correlation with dissolved P in runoff and a high level of reproducibility when results from participating laboratories were compared (RSD

<11%). The 100:1 ratio allows for reasonable and representative subsampling of diverse manure and biosolids types using labware that is readily adapted to a commercial laboratory setting.

Thus, the following general protocol is recommended:

1. Manures and biosolids are analyzed “as is” to ensure that they represent material properties as they are land applied.
2. Materials are extracted on a dry weight–equivalent basis with sufficient deionized water to achieve a final extraction ratio of 100:1 (solution:solids, where solution includes liquid already in material and deionized water). For materials with a solids content <1%, do not add deionized water.
3. Shake for 1 h.
4. Centrifuge (1500 × g) and, if necessary, filter supernatant (Whatman #1).
5. Determine extract P by colorimetry or ICP and express on a dry weight–equivalent basis.

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